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(54) Title: ULTRAVIOLET PURIFICATION OF BIOLOGICAL FLUIDS, BLOOD SERA AND OTHER CONTAMINATED SOLUTIONS

(57) Abstract

A method for the sterilization and purification of contaminated biological fluids and sera comprises the exposure of said fluids or sera to ultraviolet light of a specific wavelength below about 250 nm. The ultraviolet light is emitted from a xenon, ion laser, deuterium or mercury lamp source and is preferably tailored to a specific narrow wavelength size range that corresponds to that which optimally inactivates and/or kills the virus bacteria or other microorganism of concern. The ultraviolet light is focused as a controlled beam and by specifically calibrating the exposure wavelength with other parameters such as energy intensity, exposure time and depth or amount of fluids treated, the contaminant virus or microorganisms can be effectively destroyed without affecting proteins and serum or fluid components.

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ULTRA-VIOLET PURIFICATION OF BIOLOGICAL FLUIDS, BLOOD SERA AND OTHER CONTAMINATED SOLUTIONS

FIELD OF THE INVENTION

The present invention relates generally to the purification and/or sterilization of therapeutic fluids, cellular-produced pharmaceuticals and other related therapies. More specifically, the present invention relates to the *in vitro* sterilization and purification of blood-derived products such as serum, plasma and other mammalian body fluids as well as drug products produced by cellular cultures through the exposure of same to ultra-violet light.

BACKGROUND OF THE INVENTION

Notwithstanding the advances in modern medicine and biotechnology, there is an ever increasing a need for improved analytical testing methodologies and purification methods of human and animal blood sera, downstream cellular-derived pharmaceuticals and other biotechnology-driven technologies. With the advent of human immunodeficiency virus (HIV) and other often fatal, highly infectious viral and bacterial diseases, the need to sterilize and/or purify whole blood and plasma used in transfusions and other therapies is of great importance in both the human and veterinary medical sciences. Despite the diligent efforts of modern medicine, contaminants of blood, plasma and cellular-derived drugs more often than not go undetected and surprisingly, in many cases, no tests even exist for some known contaminants.

Clearly, a technology that eliminates all pathogens known and unknown in human sera and cell derived therapeutics would be of great value to medicine. Of even greater value would be a means to sterilize and/or purify human and animal therapeutics without also destroying or inactivating the non-

contaminating elements as well.

Scapies, Creutzfeld-Jacob Disease (CJD), Bovine Spongiform Encephalopathy (BSE) and Kuru are all believed to be caused by an agent that exhibits characteristics similar to both viruses and proteins. They have been classified as Transmissible Spongiform Encephalopathies. These agents are nearly impossible to inactivate in biological fluids without the total destruction of the fluid. Recently it has been documented that BSE has been passed to humans via consuming contaminated beef and beef byproducts. The disease is progressive as it affects the central nervous system by forming plaques in neural tissue (brain) and is 100% fatal within one year of onset. There are no known cures or effective diagnostic tests. This disease poses a tremendous threat to human and animal derived blood and tissue derived products used as therapeutic agents.

There have been numerous attempts to decontaminate biological fluids chemically, mechanically and by using radiation of various types. The use of solvent/detergent mixtures and various forms of heat treatment to inactivate viruses and other microorganisms has been widely used to prepare various blood derivatives. Most, if not all of these chemical treatments raise toxicological concerns however, as it is difficult, if not impossible, to prevent interaction of the reagents with the native red blood cells, platelets and plasma proteins as well as insuring all of the chemical is removed or deactivated from the sera prior to its therapeutic administration.

United States Patent No. 5,413,758 to Campbell et al. discloses a process and related equipment for plasma sterilization which entails injecting the plasma into a chamber which is then pulsed with an antimicrobial agent and pressurized for

a pre-determined period of time. The antimicrobial agent is carried in an inert carrier gas both of which are removed by evacuation of the sterilization chamber.

United States Patent No. 5,418,130 to Goodrich et al. discloses and claims a method for the inactivation of viral and bacterial blood contaminants. The blood cellular matter and/or plasma is first mixed with a chemical sensitizer such as quaternary ammonium or phosphonium-substituted halo-psoralen compounds. The biological sera fractions are then irradiated with ultraviolet (UV), visible, gamma or X-ray irradiation which destroys the contaminants as they were rendered unstable by the halo-psoralen sensitizers.

United States Patent No. 5,342,752 to Goodrich et al. discloses a method for inactivating viral, bacterial and parasitic contaminants in cellular blood matter or plasma blood protein fractions. Photo-chemical sensitizers such as acridine dye and/or its derivatives are selectively bound to single and double-stranded DNA of the microorganism which is then irradiated with ultraviolet or gamma radiation and effectively destroyed.

United States Patent No. 5,176,921 to Corash et al. discloses a method for the purification of biological sera by removing functional polynucleotides. The sera is first treated with one or a number of psoralin derivatives in combination with glucose and then irradiated with ultraviolet light. The biological sera is allegedly decontaminated of any viruses and any polynucleotides are rendered inactive by the UV light while the serum proteins retain their original physiological activities.

United States Patent No. 5,133,932 to Gunn

et al. teaches a method for the purification of blood and other biological fluids through the sterilization and destruction of any bacteria, viruses, parasites and other macromolecular components by treating a thin layer of the fluid with ultraviolet radiation.

United States Patent No. 5,104,373 to Davidner et al. teaches the extracorporeal treatment of certain blood-borne infections such as HIV using an apparatus that heats the sera, mechanically shears it and radiates it with UV light, X-ray or laser radiation. Heparin may be first administered to the patient's blood system so as to induce lymphocyte migration from the lymph system to the circulatory system prior to the blood's removal. The blood is then treated and returned to the patient.

United States Patent No. 5,041,537 to Behtke et al. teaches a commercial scale method for preparing biologically active transferrin in which blood sera is fractionated and the beta-globulins separated from the fraction containing the transferrin. This is subjected to ultra-filtration, ion-exchange chromatography and combined with beta-propiolactone, is further subjected to UV radiation thereby producing a biologically active, virus-free transferrin compound.

United States Patent No. 4,960,408 to Bisaccia et al. discloses the treatment of HIV-infected blood by administering a psoralen compound to the patient's blood followed by treatment with UV-light or electromagnetic radiation. The activated psoralen compound attaches the free virus or virus-infected cells in the blood and this in turn stimulates an effective immune response against the infection.

United States Patent No. 4,727,027 to Creagan et al. discloses a photochemical decontamination treatment of whole blood or plasma in

which the blood is first mixed with furocourmarin compounds followed by irradiation with UV-light or gamma-radiation. It is asserted that the normal blood proteins retain their structure and physiological activity while the contaminants are deactivated and/or destroyed. Reduction in the amount of dissolved oxygen in the blood inhibits denaturation of the proteins.

Finally, United States Patent No. 3,926,556 to Baucher discloses a method and apparatus for the low temperature intermittent or continuous destruction of viruses, bacteria and fungi in biological and organic fluids wherein the fluid is subjected to UV energy with a wavelength of from about 40 angstroms to about 3100 angstroms combined with microwave energy having a wavelength of from about 1-25 cm while the temperature is maintained below about 100°C.

None of the prior art processes and apparatus employed therein afford a complete purification and/or sterilization of the biological fluids in which all viruses, bacteria, other pathogens and polynucleotides are effectively destroyed and/or inactivated without affecting the structure and physiological function of the cells, proteins and other components of the biological sera in its uncontaminated state. Moreover, none of the prior art discloses a method for the purification or inactivation of biological sera, vaccines, downstream drug products derived from cellular and microbial sources and other contaminated fluids such a waste water in a simple, cost effective manner on a large scale basis.

SUMMARY OF THE INVENTION

The present invention provides a method for the sterilization and purification of biological sera and other contaminated fluids through the deactivation

and killing of viruses, bacteria and other pathogens and their polynucleotides througout the exposure thereof to a precise spectra of ultraviolet wavel ngth radiation. The precisely controlled wavelength radiation is specific for the molecular make-up of the contaminant cell and thereby destroys it leaving the surrounding proteins, cells and other naturally occurring components intact.

DETAILED DESCRIPTION OF THE DRAWINGS

Figure 1 is an over-all schematic side view of the ultra-violet irradiation-light source and fluid cell.

Figure 2 is an isolated side view of the exposure cell.

Figure 3 is a schematic side view of an ultraviolet irradiation unit with a fiber optic cable.

DETAILED DESCRIPTION OF THE INVENTION

Light is wave energy that is commonly subdivided into spectral regions. The wavelength of a particular source refers to the distance between two peaks. Humans are only capable of visualizing the longer wavelengths, however light which is invisible to humans transmits various energy characteristics as well. Ultraviolet light generally refers to the spectrum between infrared energy and X-rays. Ultraviolet light is often subdivided into three regions - A, B and C, whereby C refers to the shortest wavelength region. Common scientific references to UV light used in inactivation methods may usually be translated as meaning approximately 254 nanometers (nm), the predominant emission of a mercury lamp, the most commonly used source for UV light.

Ultraviolet light at or near 254 nm is absorbed by the bonding structure of nucleic acids. Nucleic acids are the backbone of genetic material,

RNA and DNA. This phenomena has been exploited in the detection and quantification of nucleic acids in solution via widely used spectrophotometric techniques and instruments (spectrometers). At higher energy levels, UV light has been widely documented to destroy the nucleic acid bonds, thereby inactivating exposed microorganisms by disrupting their ability to reproduce or transfer the genetic material. Viruses are largely composed of genetic material in the form of DNA or RNA, double and/or single stranded, that is associated with a protein or lipoprotein coat. UV light disrupts the DNA and/or RNA and has been found to be most effective on single-stranded structures although it is also effective on double-stranded structures to a slightly lesser degree. The known or suspected bond destruction mechanisms are: hydration of the pyrimidines; the rupture of hydrogen bonds, the initiation of breaks in the phosphate bonds pyrimidine dimerization, pyrimidine photoadduction and the photoadduction of amino acids to the nucleic acid strand

Whereas ultraviolet radiation has been used previously in an effort to purify and sterilize biological sera and other fluids, these methods have generally required the additional incorporation of chemical sensitizers and other chemical agents to effectuate any reduction in the viral, bacterial and other pathogenic elements. Moreover, the source of UV-radiation in the past has traditionally been from a mercury lamp alone and as result, wavelengths used to destroy the microorganisms and other polynucleotides has been in the 254 nm range. Unfortunately, at this wavelength, the UV light also destroys many valuable proteins, amino acids, enzymes, cells and other valuable components that comprise the normal uncontaminated biological sera or fluid. This is partly because the light spectrum at which the

ultraviolet wavelengths are transmitted include wavelengths of infrared radiation which are the major source of radiant heat or incident heat produced by the light source that destroys cells and heat labile proteins. Moreover, the formation of ozone mediated by UV exposure to oxygen in the proximity of the fluid can also destroy valuable components of the fluid.

Some proteins are known to absorb UV energy at or above 254 nm with peak absorption recognized as being near 280 nm. Exposure of proteins to high energy at 254 nm has also been documented to result in protein destruction or denaturization thereby adversely affecting their inherent biological activity. Therefore, the devices known in the art used to inactivate viral and bacterial contaminants in biological products such as sera will also break down many of the sera's useful components. The present invention then focused on the lower wavelengths within specific frequencies that will destroy the contaminants yet leave the biological sera components (such as lymphocytes, red blood cells, platelets, antigens, Factor VIII, etc.,) in blood intact. This yields a truly functional sera that is purified and free of contaminants for administration to patients and others in need.

The present invention comprises an apparatus that produces high intensity variable wavelengths within the ultraviolet A, B and C spectra with a calculated filter and photometry grid. Using a xenon, deuterium, ion laser or mercury lamp as a light source, the device is able to produce ultraviolet wavelengths with energy levels in a range of from about 170 nm to about 250 nm. The method employed selectively inactivates and/or destroys specific viruses, bacteria, transmissible Spongiform encephalopathies (TSE's), mycoplasma, prions, polynucleotides, fungi, yeast, endotoxins,

polypeptides and other macromolecular contaminants by varying the ultraviolet wavelength, intensity, exposure time and/or pathlength of th exposure cell.

The present invention is based upon the fact that viruses and proteins respond differently to the exposure of different wavelengths of ultraviolet energy. It is well known that UV energy and the lights' wavelength are inversely proportional. Light is a form of wave energy and the wavelength of light refers to the distance between two peaks. As the wavelength decreases, more energy is possessed by the photons due to the increased frequency of wave peaks. It has been demonstrated that nucleic acids, DNA and RNA, will exhibit a greater destructive response to shorter wavelengths.

The target for the UV radiation of the process of the present invention are the free viruses, bacteria and other microorganisms that are not associated with the fluids' native cells which therefore do not have access to the cell-mediated repair mechanism. It has been shown that exposure to a wavelength of 248 nm generated by a continuous wavelength laser had an increased destructive effect on nucleic acids. These destructive effects have been characterized as formation of photodimers, photohydrates and other adducts. This suggests there may be an optimum wavelength window where an optimum wavelength exists where optimum nucleic acid kill can be achieved and optimum protein conservation observed.

The virus and protein assays are quantitative, therefore, by exposing the virus and/or protein to a power level that did not provide complete inactivation, the plotted results create a wavelength dependent dose/response curve. That is, the effects of altering the exposure wavelength at a constant power show that the lower wavelengths are more destructive to the virus. In the case of the protein,

it is a bit more complex, since protein inactivation is dependent to a great extent on the proteins' content of "aromatic" amino acids such a tryptophan, tyrosine and phenylalanine.

The apparatus and method for its use then capably purifies and sterilizes biological sera of a wide variety of contaminants while at the same time causing no harm to the cells, proteins, blood factors and other cellular and sera components by setting specific UV wavelength and energy parameters below 254 nm. By eliminating the longer ultraviolet wavelengths, i.e. greater than 250 nm, the method and apparatus of the present invention eliminates the higher levels of heat or ozone generated thereby which would further cause damage to the biological sample.

The contaminated biological sera or fluids are UV-radiated in a closed exposure cell which prevents air or other gases from contacting the sample thereby insuring oxygen will not affect the photochemical reactions. The specific UV radiation exposed to the sample can also be calibrated to destroy specific viruses, macromolecules and other microorganisms since the smaller wavelengths, i.e., below 250 nm, actually are of higher energy levels than the longer wavelengths above 250 nm and hence will have a greater effect over shorter durations.

The present invention may be used in a wide variety of applications. The UV radiation of the specifically tailored wavelength and parameters may be used to treat animal-derived products, in particular blood-derived agents, sera and plasma, fluids useful in the manufacturing of pharmaceuticals, biopharmaceuticals, vaccines and other sera as well as cell culture products, the purification of water and extracted therapeutic compounds.

The tailored UV wavelength spectra is perhaps most importantly useful in the treatment of

human derived blood products. This application is driven by safety concerns such as the transmission of hepatitis, HIV, AIDS and other pathogens in the blood, plasma and other body fluids. The ability to destroy or deactivate the specific virus, bacteria, or other pathogenic microorganism without affecting the blood cells and plasma components will go a long way in insuring the safety and integrity of the world's blood supply.

The apparatus and method of the present application is also useful in the purification and/or inactivation of viruses and mycoplasma often found in genetically engineered drugs extracted from cell cultures. Since all biotech related pharmaceutical manufacturers must show the Food and Drug Administration (FDA) a minimum log reduction in viable virus particles per estimated final administered dose for a particular therapy in any IND or NDA before clinical approval, this application of the present invention has wide ranging value. Whereas the effects of UV-radiation on biological materials are well documented, the present quantitative technology which possesses the potential to deliver a selective narrow ultraviolet wavelength at sufficient energy levels within measurable parameters will have significant application in pure research. In this manner, the method and apparatus may also prove useful in drug design and manufacturing processes as well as the sterilization of otherwise heat labile compounds, chemically sensitive compounds or those not suited to standard filtration methods.

The apparatus and method of the present invention is also useful in the purification and sterilization of waste water and polluted water samples for re-use in industry and by humans. Filamentous blue-green algae, viruses and other microorganisms cannot be easily removed by most

standard filtration devices but when used in conjunction with the present invention a clean, re-usable source of water is obtained.

The biological sera are irradiated with ultraviolet radiation with wavelengths in the spectral range of from about 200 nm to about 250 nm. Within this broad range, the wavelengths are then calibrated to specific narrow ranges that optimally kill or destroy the contaminant(s) of concern while creating the least amount of risk to the surrounding sera and cells. The wavelength then, is specifically tailored to that value which destroys the cell wall, membrane, protein structure or whatever of the particular contaminant sought to be removed.

The specific wavelength that provides optimal kill or inactivation of each virus, bacteria, or other microorganism of interest is determined within a narrow range of from about 3.0 nm to about 10.0 nm within the broader UV spectra of 200 nm to 250 nm and preferably within a range of 3.0 nm to 5.0 nm. The absolute ultimate wavelength selected may vary along with the other variable parameters, all of which are mutually dependent upon one another. It is critical then, that the inactivation energy delivered to the sample be coordinated with the other parameters of exposure time, sera/fluid flow rate, and light intensity so that the specific organism is killed without denaturing the native blood proteins and other cellular components.

The collimated or manipulated UV beam is focused on an exposure cell which contains or through which flows the biological sera to be irradiated. Purification and decontamination may be carried out in a batch style format using a containment vessel for the exposure cell or preferably, through a tube or feed line in which the biological sera is pumped and inactivation/desterilization and purification is

carried out on a continuous basis. Generally, batch exposures will utilize lower energy levels and longer exposure times while the continuous method will utilize more intense UV-radiation energy for much shorter exposures per unit exposed in the cell. The continuous flow method is preferred and scaled up configurations can treat more than twenty (20) liters/hour.

The duration of UV-radiation exposure to achieve inactivation is dependent on the exposure conditions supported by the configuration of the exposure device which determine critical parameters. Specifically the parameters are fluid depth, energy distribution, wavelength and the fluid's ability to absorb UV energy. Exposure in a stationary configuration may range from many seconds to minutes or longer. In a continuous exposure process, any particular unit of the biological fluid will be exposed for a short interval as the fluid passes through the exposure cell. The fluid may also be passed through the exposure cell multiple times or through exposure cells arranged in a series to create the appropriate inactivation conditions.

The UV lamp may also be calibrated to deliver multiple wavelengths simultaneously. Two narrow UV wavelengths will assure killing of more than one specific virus or bacteria. The multiple wavelength delivery feature may permit selective killing of two types of organisms while limiting its effects on the other surrounding cells and proteins.

The exposure cell itself is made out of a clear material that will not appreciably block, reflect, absorb or refract the UV beam. Generally, quartz, sapphire or UV grade fused quartz silica cells provide excellent transmission of UV light. The cell is then coated with an exposure cell window transmission material such as polytetrafluorocarbon

(PTFE), commercially available under the tradename Supertrans®. This allows the UV wavelength to pass through unadulterated. This material is of particular value in the present application as it is also unwettable and thereby prevents any build up of protein and/or cellular debris on the window surface which could ultimately affect ultraviolet light transmission and performance.

The cell thickness or exposure depth can also be adjusted. This provides some latitude in designing processes and gives the user control to precisely determine the optimum wavelength in each application. In the present invention, the fluid thickness is determined by the physical restraints of the pathway which is dictated by the distance between the exposure cell plates which is maintained with spherical beads or spacers of a specific thickness. In the Free Surface design, fluid thickness is created and maintained by managing the shape, flow rate, angle, and fluid delivery mechanisms of the exposure cell. An inactivating dose is achieved by delivering a specified minimum power density of UV energy per unit volume of fluid. Therefore, all structures targeted for inactivation must be exposed to the minimum power necessary for inactivation to occur. This can be portrayed in terms of Joules per cubic centimeter or minimum Joules per square centimeter throughout the entire depth of the sample fluid.

The exposure cell is an air-tight enclosure, i.e., it is not exposed to the atmosphere which means that it can be internally maintained in a sterilized condition. The cell is also manufactured from materials that are insensitive to heat and steam i.e., autoclave conditions so that after each use it can be steam sterilized. The materials that comprise the cells' construction are also resistant to harsh chemicals and high or low pH conditions commonly used

to chemically sterilize lab equipm nt.

In the past, the optimum UV penetration depth has been reported to be approximately 0.3 mm. This is extremely thin, about the thickness of a business card. There has been other research that indicates a thickness of 4 mm can be effectively irradiated. The actual optimum exposure cell thickness for the UV instrument of the present invention can be increased by illuminating the top and bottom surface of the exposure cell thereby doubling the penetration achievable. A reflecting back surface of the exposure cell can also constructed to "catch" reflected UV energy and pass it back through the sample thereby nearly doubling the inactivation potential. This may be complicated by the absorption characteristics of the carrier fluid however.

In another embodiment, the incident UV irradiation can be focused or reflected from other surfaces of the exposure cell, much like a tanning reflector is used to concentrate the sun's rays on the face. In still a third embodiment the UV light can be concentrated via a lens assembly. The light source (lamp) may be located in a box separate from the exposure cell housing. The desired wavelength is filtered and subsequently focused into a fiber optic cable. The energy can be transmitted to the exposure cell through the cable and expanded or focused by using lenses to deliver a high energy beam in a precise shape or configuration.

The exposure cell may be designed then in one of three configurations:

a. A free surface that is flat in relation to the light source. In this design there is no transparent surface covering the exposure cell. This is the most efficient design and would eliminate the formation of UV-light retarding protein debris that may accumulate on the exposure surface. It is

difficult however, to form and control a thin film on an open, free surface.

b. Preferably, the cell may be constructed in a sandwich design whereby two plates of UV transparent material (quartz, sapphire, plastic or UV grade fused silica) are separated by a space consisting of precise spherical particles displaced throughout the plate or spacers aligned on the outside edges of the plates. The fluid or sera flows between the plates and is exposed to the UV irradiation from the top surface with a reflective backing on the bottom surface for maximum exposure. In a second embodiment, the bottom surface might also be transparent whereby the fluid is exposed to a second source of UV-irradiation from below and is therefore irradiated from both the top and bottom surfaces.

c. The cell may also be constructed as a sandwich design as in (b) with a UV transparent disposable lining consisting of Teflon® or other UV transparent plastic polymer. This design eliminates the concerns regarding cleaning and potential for cross-contamination between runs. The plastic polymer bag is provided pre-sterilized.

The closed cell also does not permit ozone (generated naturally via UV-irradiation contact with O_2 or air) to come into contact with the sample. This is important because ozone (O_3) is a destructive gas that destroys both proteins and viruses. Eliminating ozone from contacting the sample helps conserve the proteins in the sample solution. As a further safeguard, nitrogen gas (N_2) is frequently injected into the chamber to purge any ozone that might inadvertently be formed and enter the cell.

The xenon, deuterium, ion laser or mercury lamp is incorporated within a housing with a standard transmitter/regulator, grating or other optical filter which allows for wavelength size control and

variation. Wavelength intensity is a function of the energy output of the UV lamp and is raised by changing the voltage to the lamp and control of same is provided using a transformer and voltage regulator as is known in the art.

Ultraviolet light filters are also well known in the art and are commercially available from a variety of sources. The filter may be interchangeably used to also vary the beam intensity and focus and may also be used instead of a variable transmitter to change the intensity of the UV beam in order to focus it on the nucleic acid structure of the organism or molecule of interest. The filters are placed between the light source and the exposure cell and this serves two additional functions. The filter absorbs and blocks the harmful infrared wavelengths that generate the radiant heat that ultimately damages the surrounding cells and proteins of the biological fluid. In another embodiment, the upper surface of the cell can be comprised of the light filtration material thereby obviating the need for a separate filter unit. The filter is also tilted at an angle of approximately 10° with respect to the path of the beam so that any incident infrared rays and the inherent heat therein are reflected back away from the cell but not into the lamp. Thus, only the variable ultraviolet wavelengths of a narrowly defined size and intensity reach the sera.

The exposure cell itself is also constructed with a reflective back surface that reflects nearly 100% of the UV light beam back through the sample. Hence, the sera is essentially treated twice when pumped through in a continuous mode. Ultraviolet light sensors can also be incorporated within the cell to insure the intensity of the beam is correct. Level sensors can also be added to insure the angle of the cell surface is proper.

The apparatus of the present invention is designed so as to enable the continuous inactivation of a flowing stream of fluid. The biological fluid is exposed to the UV light in an exposure cell that channels the flowing fluid stream into a thin film for minimum inactivation for a short period of time. The instrument is also capable of monitoring fluid exposure conditions such as the degree of exposure power, residence time, flow rate, wavelength exposure and the like. Should any of these parameters fluctuate from the programmed norms, a control unit allows for the immediate shut down of the operation, the activation of alarms or the diversion of the fluid flow.

Exposure conditions, i.e., residence time, wavelength, etc. will vary when applied to different virus/protein mixtures found in biological sera such as blood. Hence, precise parameters must be derived for each virus/protein combination encountered.

Referring now to Figure 1, the ultra-violet fluid purification device (2) of the present invention is schematically represented and is comprised of the broad spectrum ultra violet light source (4) that consists of the xenon, deuterium, ion, laser or mercury lamp. In close proximity thereto is located the lens/filter assembly (6) which filters out or removes those UV wavelengths which generate heat that would otherwise be detrimental to the desirable native proteins, blood cells, etc. The lens is selectively controlled to focus the ultraviolet light to the desired wavelength and intensity. The light then passes through a second lens/filter assembly (8) which also may be calibrated and ultimately emits the UV light in the desired wavelength to the exposure cell (10). UV light sensors (9) disposed along the surface of the cell monitor the intensity of the beam (13) to insure the correct calibration of the wavelength

intensity. Level sensors (11) insure the cell surface angle is correct with respect to the direction of the beam. The embodiment as set forth in Figure 1 also shows the optional design wherein a second UV light source (14) is located below the cell with a second lens/filter assembly (12) so that the sample is exposed to the radiation from both sides of the cell.

As seen in Figure 2, the exposure cell (10) is constructed in an essentially three layer, sandwich assembly comprised of suitable UV transparent material such as quartz or sapphire. Sandwiched between the cell walls is the channel for the flow stream (15) of biological sera to be purified. Optionally, a disposable, transparent bag or liner (16) may be disposed therebetween in order to facilitate cleanup after use and to help protect against cross-contamination of sera. This also helps to insure the flow stream remains free flowing and not clogged by cellular debris, proteins and other serum matter that may collect on the inner surface of the channel over time.

Figure 3 is a schematic representation of the UV biological sera purification system of the present invention with the optional fiber optic cable and expansion lens design. The UV light source (4) and the lens/filter assembly (6,8) focus the light to a fiber optic cable coupling junction (18) which then feeds the light through a fiber optic cable (19) to a second fiber optic coupling unit (20). This second coupling unit transmits the ultraviolet light or laser energy through an expansion lens (22) which disperses the light over a wider surface area than that of the initial beam restricted by the minute circumference of the fiber optic cable (19). The expanded UV light particles then irradiates the exposure cell surface (10) in the same manner but in a much heightened intensity as before. This embodiment also shows how

the UV source and exposure cells are encased within separate housing (24) to insure a sterile environment and to protect the instrumentation from external disturbance.

The following examples are set forth to better describe and disclose the inactivation/purification process and apparatus of the present invention. They are for illustrative purposes only, and it is recognized that a number of changes and alterations may be made with respect to the device of the present invention and the parameters employed in its use which have not been specifically discussed herein. It should be understood that any such changes that do not materially alter the apparatus or its' use in the sterilization and purification of biological sera and fluids are still considered as falling within the spirit and scope of the invention as recited by the claims that follow.

Example I

Various UV wavelengths were exposed to fluids in order to demonstrate their ability to inactivate specific viruses and proteins. The exposure conditions are designed to evaluate the effect of varying wavelength.

A 70 cm diameter round exposure cell constructed of UV Grade Synthetic Fused Silica glass was constructed. The sample solution (containing a known concentration of virus and/or protein) was sandwiched between two glass plates with a spacer of 0.3 mm thickness. This provided an experimental sample volume of 1 ml. The 0.3 mm space has been regarded as being the optimum thickness that UV light will penetrate (See Sing Chin et al. Blood Vol. 86, No. 11, Dec. 1, 1995 pp 4331-4336 which is hereby incorporated by reference). The present design utilized a thickness of 0.3 mm to insure complete

exposure of the sample.

Virus Samples	Protein Samples
MuLV - Murine Leukemia Virus	Insulin
Pseudorabies	Interferon
Poliovirus	Trypsin
Parvovirus	Peroxidase
Reo -3	
IgG - Immunoglobulin	

Exposure wavelengths were generated by a Coherent Frequency Doubled Ion Laser System (300 FreD). The following wavelengths were evaluated (1)257.2, (2) 248.2, (3) 244.0, (4) 238.2 and (5) 229.0.

The energy level was adjusted so that less than 100% inactivation of the sample(s) were achieved. It was found that power levels of 0.1 Joules to 0.7 will inactivate the specific viruses. The delivered power for each of the samples remained constant, only the wavelength differed for each sample. The power dependent response curve generated from this experiment showed that as the power is increased the level of inactivation for viruses is increased and the negative effects on the proteins is not substantially affected.

Example II

Experiments were designed to demonstrate the present invention's ability to inactivate viruses and a model protein molecule in a flowing stream of fluid. The instrument consisted of high intensity xenon arc lamp, a UV-light filtering assembly and a continuous flow cell. The samples were flowed through the exposure cell receiving a UV wavelength exposure ranging from 200nm to 250 nm. The power was adjusted to attain 0.05 Joules to 0.35 Joules exposure. Optical surfaces were coated with a proprietary,

inert, non-stick substance (Supertrans) to prevent the accumulation of debris on the fluid contact surfaces. The experimental thin film consisted of an aqueous solution containing a known quantity of virus and/or protein. After continuous flow exposure the sample fluid was collected in fractions. A wash cycle followed each exposure to insure that no virus remained in the fluid lines. Control samples were also collected and the virus/protein solution passed through the same pathway with the UV source turned off. The exposed samples and controls were then evaluated for virus and/or protein activity (viruses - quantitative plaque assay; protein - enzymatic activity).

A. **Poliovirus**

Polio virus is from the Picornoviridae family, approximately 30 nm in diameter, ssDNA (single stranded DNA nucleic acid content), non-enveloped and it demonstrates high physicochemical resistance to inactivation. This virus was chosen because of its relative resistance to inactivation by current methods and its potential significance as a serious pathogenic threat.

The experiment, conducted in triplicate, demonstrated complete inactivation of the virus loaded in the test sample at three power levels ranging from 0.1 to 0.2 Joules. The minimum inactivation was shown to be greater than 7 logs of virus particles per ml.

B. **Parvovirus**

Parvovirus is from the Parvoviridae family, approximately 18-26 nm in diameter, ssRNA, non-enveloped and characterized as possessing a medium physicochemical resistance to inactivation. This virus was chosen for its extreme resistance to gamma irradiation and the fact that it is the smallest known

virus. It is widely distributed and often detected in animal and human-derived products. Due to its physical characteristics, there are few existing options regarding the inactivation of this virus.

As in the poliovirus experiment, it was conducted in triplicate. The results showed complete inactivation of the virus load of greater than 7 logs of virus particles per ml. The samples were exposed to UV-light energy in the power range of 0.1 to 0.18 Joules.

Example III

Horseradish peroxidase is an enzyme that is extensively used as a reporter molecule in quantitative enzyme mediated protein experiments such as ELISA. The enzyme reacts with TCB (a reagent) resulting in a calorimetric reaction indicative of the amount of enzyme present. The less intense the color, the less active enzyme present in the solution. Measurements were carried out in a spectrophotometer (Taktagen, Inc). The procedure, reported in the article entitled "Inactivation of the Agents of Scrapie, Creuizfeldt-Jakob Disease and Kuru by Radiations taken from Slow Transmissible Diseases of the Nervous System", 1. 2 1979 Academic Press ISBN: 0-12 566302-1 which is hereby incorporated by reference, was followed and showed that the causative agents of Scrapies responded in the same manner as peroxidase to exposure to UV irradiation. In other words, the inactivation response of Scrapies and peroxidase are quantitatively the same. Specifically, the Scrapies agent responded progressively better (exhibited greater levels of inactivation as the exposure wavelength decreased. There was negligible destruction of the causative agents at 254 nm, i.e., the emission from a mercury lamp. As the exposure wavelength was decreased to 220 nm, a 33-fold increase

in the destructive effects of the UV radiation was observed. Hence, the present invention is capable of delivering the required power at the lower wavelengths.

The peroxidase experiment was designed similar to the virus experiments. A continuous flow of peroxidase solution was exposed to the UV light in the exposure cell. A control solution containing approximately 1 mg/ml of peroxidase was made. The control solution was analyzed for peroxidase activity after passing through the instrument with the UV light source turned off. Three samples drawn from the control solution were irradiated at 0.34 Joules. The assay results showed that the peroxidase activity was inactivated by 44%, 41% and 60% respectively, after exposure.

Horseradish peroxidase (HRP) enzyme was prepared in four dilution's; 1 mg/ml, 0.1 mg/ml, 0.05 mg/ml and 0.01 mg/ml. A portion of each solution was passed through the instrument with the light turned off to serve as experimental controls. The other portion of the same HRP dilution's were then passed through the instrument, irradiated, collected separately and analyzed for enzyme activity. The assay results showed that peroxidase activity was reduced by 88%, 57%, 78% and 80% respectively. This observation was considered significant in demonstrating that the UV instrumentation is capable of delivering the proper irradiation conditions necessary to inactivate TSE's in a flowing biological fluid stream.

What is claimed is:

1. A method for the purification and sterilization of contaminated biological fluids and sera comprising the exposure of a sample of said fluids or sera to a beam of ultraviolet light of a narrowly defined wavelength that is less than or equal to 250 nm for a sufficient period of time.

2. The method of claim 1 wherein said biological fluid is selected from the group consisting essentially of, plasmas, serum and blood derived products, biopharmaceuticals, pharmaceuticals, vaccines, protein solutions, water and mixtures thereof.

3. The method of claim 2 wherein said bean of ultraviolet light is calculated to a specific wavelength that is capable of inactivating a specific virus, bacteria, fungi, TSE, yeast, prion or other microorganism without denaturing the constituent native proteins, polynucleotides, cells and other components that comprise the sera or fluid.

4. The method of claim 3 wherein said ultraviolet light is emitted from a xenon, deuterium, ion laser or mercury light source.

5. The method of claim 4 wherein said ultraviolet light source is calibrated to a wavelength of from about 200 nm to about 250 nm.

6. The method of claim 5 wherein said ultraviolet light is of a specific wavelength that may vary within a range of from about 1.0 nm to about 10.0 nm.

7. The method of claim 6 wherein said ultraviolet light is of a specific wavelength that may vary within a range of from about 1.0 nm to about 5.0 nm.

8. The method of claim 7 wherein said ultraviolet light source may be calibrated so as to allow for the variance of the ultraviolet wavelength to a specific value between 200 nm and 250 nm.

9. The method of claim 8 wherein said biological fluid or sera is exposed to said beam of ultraviolet light in a self-contained exposure cell.

10. The method of claim 9 wherein said biological fluid or sera is exposed to said ultraviolet light beam in a single batch mode.

11. The method of claim 9 wherein said biological fluid or sera is exposed to ultraviolet light during multiple contacts in a continuous flow mode.

12. The method of claim 9 wherein said sera or fluid is exposed to multiple UV wavelengths simultaneously.

13. An apparatus for the sterilization, purification and inactivation of viruses, bacteria, prions, polynucleotides and other pathogenic microorganisms present in a sample of a biological fluid or sera without affecting the surrounding native proteins, polynucleotides, cells or components thereof comprising:

a. a light source for the emission of ultraviolet light at wavelengths below about 250 nm;

b. a lens for focusing said

ultraviolet light as a controlled beam to an exposure cell containing a portion of said biological fluid or sera;

c. a filter for the removal of heat generating infrared radiation;

d. a means for calibrating said ultraviolet light to a narrowly defined wavelength at a specific energy intensity; and

e. a means for continuously flowing said biological fluid or sera through said exposure cell for a time sufficient to kill or inactivate said viruses, bacteria or other microorganisms.

14. The apparatus of claim 13 wherein said UV light is emitted from a xenon, deuterium, ion laser or mercury light source.

15. The apparatus of claim 14 wherein said ultraviolet beam is further passed through an arrangement of photometric filters and photometric grids prior to contacting said exposure cell.

16. The apparatus of claim 15 wherein said wavelength is from about 170 nm to about 250 nm.

17. The apparatus of claim 16 wherein the wavelength of said ultraviolet light is calibrated to specific range that may vary between about 3.0 nm to about 10.0 nm.

18. The apparatus of claim 17 wherein said wavelength is calibrated to a specific size range that may vary from between about 3.0 nm to about 5.0 nm.

19. The method of claim 8 useful in the treatment of human-derived blood sera and other bodily fluids.

20. The method of claim 8 useful in the purification of animal derived blood products used in the production of pharmaceuticals, vaccines and other therapeutic sera.

21. The method of claim 8 useful in the purification of therapeutic proteins and other genetically engineered compounds extracted from cell cultures.

22. The method of claim 8 useful in the purification and treatment of waste water and contaminated natural water sources.

23. The apparatus of claim 13 useful in the treatment of human-derived blood sera and other bodily fluids.

24. The apparatus of claim 13 useful in the purification of animal-derived blood products used in the production of pharmaceuticals, vaccines and other therapeutic sera.

25. The apparatus of claim 13 useful in the purification of therapeutic proteins and other genetically engineered compounds extracted from cell cultures.

26. The apparatus of claim 13 useful in the purification and treatment of waste water and contaminated natural water sources.

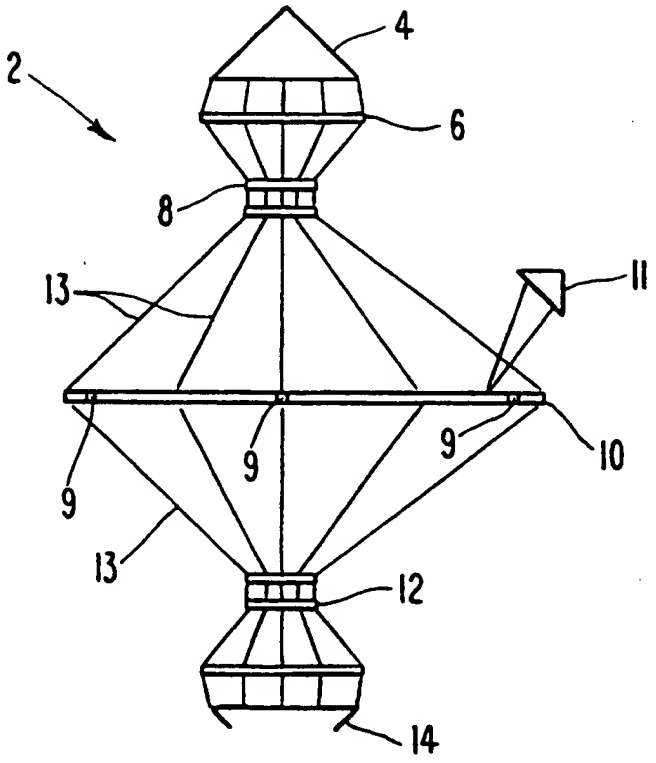

Fig. 1

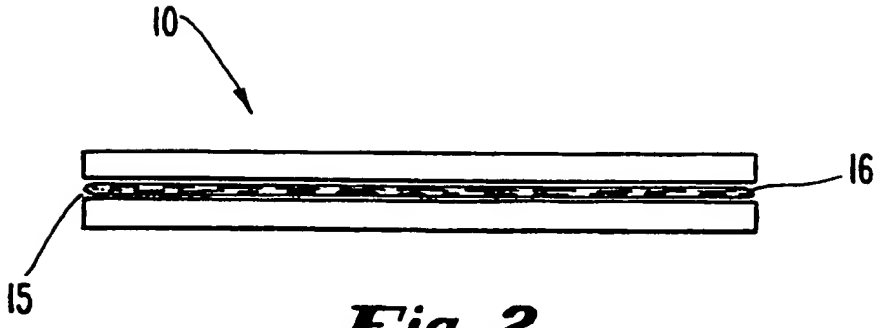

Fig. 2

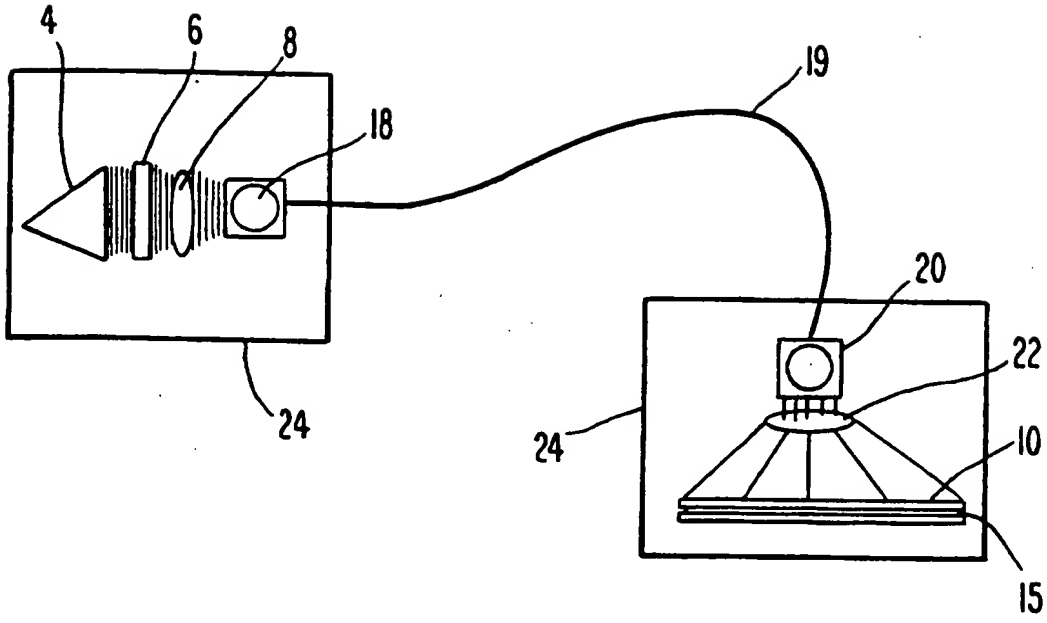

Fig. 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/04040

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :A61L 2/00
US CL :422/1, 24, 41, 44, 186.3; 250/455.11
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 422/1, 24, 41, 44, 186.3; 250/455.11

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS
search terms: UV or ultraviolet, sterilization, blood, fluids

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y	US , A, 5,133,932 (GUNN ET AL) 28 July 1992 (28.07.92), see entire document.	1-3, 5 ---------- 4, 6-10, 19-21
Y	US, A, 5,247,178 (URY ET AL) 21 September 1993 (21.09.93), see entire document.	13-18 and 23-26
X ---- Y	US, A, 5,262,066 (VAN SOYE ET AL) 16 November 1993 (16.11.93), see entire document.	1-5 -------- 6-11, 19-22
X	US, A, 4,141,686 (LEWIS) 27 February 1979 (27.02.79), see entire document.	1-3
X ----- Y	US, A, 5,376,281 (SAFTA) 27 December 1994 (27.12.94), see entire document.	1-5 --------- 6-12, 22

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 14 MAY 1997	Date of mailing of the international search report 26 JUN 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer KRISANNE M. THORNTON for Telephone No. (703) 308-0651

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/04040

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US, A, 3,926,556 (BOUCHER) 16 December 1975 (16.12.75), see entire document.	1-5 -------- 6-9, 11, 19-23